1617

HIGH TEMPERATURE–SHORT TIME STERILIZED EVAPORATED MILK. II. LABORATORY TECHNIQUES FOR THE PREPARATION AND STUDY OF STERILE EVAPORATED MILK

SUMMARY

Laboratory techniques for the preparation and study of high temperature–short time sterilized evaporated milk are described. A bomb, falling-ball microviscometer combination, easily constructed in large numbers, is employed for containing samples during sterilization and storage, and for following changes in viscosity. Normally, the instrument constant for the microviscometer is determined by standardization against liquids of known viscosity. This procedure is too time-consuming and tedious for research on evaporated milk. For such research, studies show that the instrument constant may be calculated expeditiously, with sufficient accuracy, with the help of the following equations:

$$C_M = f\left(\frac{d}{D}\right) d^2 \cos\theta; \text{ and}$$

$$f\left(\frac{d}{D}\right) = 0.0178\text{-}0.0217\,\frac{d}{D}.$$

C_M is the instrument constant, d and D are, respectively, the diameter of the ball and tube, $f\left(\frac{d}{D}\right)$ is a function of the ratio between the diameters, and θ is the angle of inclination of the tube. Forewarming and evaporation are carried out in a rotating film evaporator, and sterilization in a thermostatically controlled oil-bath. An electrically operated metronome is employed for controlling the time of sterilization. Small-scale homogenization is facilitated by the employment of a diesel nozzle tester and nozzle combination modified to function as a hand-homogenizer. The application of the techniques to the study of gelation is illustrated in studies on milks containing added calcium salts and phosphates. Wide variations in $[Ca^{++}]$ and $[HPO_4^{=}]$ influenced storage stability to only a small degree, with a small advantage accruing to the use of limited quantities of calcium salts. False body, but not the minimum viscosity attained during storage, increased markedly with increasing $[Ca^{++}]$. Repeatability of results was quite satisfactory. In one series of experiments based on the same sample of whole milk, a mean storage life of 84 days was observed; the limits for the mean at a 95% confidence level were ± 5 days.

In the production of high temperature–short time (HTST) sterilized evaporated milk, sterilizing temperatures of approximately 137° C. are employed for periods of time ranging from seconds to tens of seconds. Both the color and flavor attributes of freshly prepared HTST sterilized evaporated milk compare favorably with the corresponding attributes of market milk. Because it is capable of storage without refrigeration, HTST evaporated milk would be a worthy competitor of market milk, were it not for the fact that it tends to set to a renneted milk-like structure on short storage.

Sterile milk for gelation studies is usually manufactured on a pilot plant or plant scale. Cans of milk are opened periodically and viscosity measurements

Received for publication July 22, 1960.

are made according to accepted viscometric practice. Such studies, although they have the merit that results are immediately translatable into plant practices, have a number of disadvantages, particularly from the point of view of the chemist who would prefer to work in his laboratory dissociated from plant and pilot plant operations. In order to bring research studies on HTST sterilized milk entirely within the purview of the laboratory chemist, techniques have been developed which permit the carrying out on a small laboratory scale of many, if not all, of the processing steps: forewarming, homogenization, concentration, and sterilization. These techniques will be described in this paper and their application and advantages will be discussed. They are based on the employment of the bomb microviscometer described in the first paper of this series (4).

APPARATUS AND METHODS

Selection of tubes and beads. Certain modifications were made in the construction and operation of the bomb viscometer. For studies on evaporated milk, tubes approximately 3.5 cm. long, 0.445-0.455 cm. in bore, and 0.020% maximum bore gradient were selected on the basis of microscopic examination. Corresponding to each tube a bead was selected the diameter of which fell in the range of 0.62-0.75 times the diameter of the tube. The size range of the beads was narrowed by the use of specially constructed sieves consisting essentially of perforated brass plates about 1 in. diameter and $\frac{1}{16}$ in. thick. Numerous perforations in one plate were made with a 0.012-in. drill. Alternately, a simplified selector was used comprising the following: an open-end box constructed out of $\frac{1}{4}$-in. plastic each of the sides of which had four vertical grooves; a glass plate resting on the plastic base and hugging the sides of the box; two razor blades sliding in and out of two sets of appropriate grooves, and forming the ends of the box; a third blade the center portion of which was raised 0.012 above the glass base plate by means of strips of gauge plate, and a fourth blade the center portion of which was raised 0.011 in. The raised blades constituted partitions between which beads 0.275-0.305-mm. diameter were trapped. These beads were noticeably free from defects, inasmuch as they were representative of beads which rolled freely at a small angle of inclination. The partitions were pressed firmly against the strips of gauge plate by means of rubber bands. Clean and dry surfaces were essential for the proper functioning of the selector.

Forewarming and concentration. Forewarming of small milk samples at the temperature of boiling water for 15 min. was effected in a laboratory rotating film evaporator in the presence of air or in a current of nitrogen, as the occasion demanded. The forewarmed sample in the evaporator was concentrated in vacuo to 26% solids. Concentration was usually carried out to a point slightly beyond the desired concentration and a final volume adjustment was made with water. From knowledge of the initial solids concentration and the weight of the sample before and after concentration, the final solids concentration was controlled. Additional agitation was imparted to the contents of the flask by means of a glass baffle moving freely within the flask. Following its concentration and standardization, the sample was passed through a fine mesh wire filter screen.

Sterilization. Small samples of concentrated milk were deaerated momentarily in vacuo. Bomb viscometers were loaded with deaerated samples and mounted in fine wire stirrups. The mounted samples were plunged into an oil-bath at 137.4 ± 0.15°, and after being held for 15 sec. they were removed and plunged into water at room temperature. Timing was made by the use of an electrically operated metronome. The bringing-up time is not known with certainty; an intelligent guess based on heat conductivity theory would require about 0.5 sec. to bring the temperature at the center of the viscometer to within 0.2° of the temperature of the bath.

The oil-bath consisted of a deep fryer (located within a thermostatically controlled air bath) equipped with a stirrer and an auxiliary heater with controlled heat input operating through an electronic relay. Control of the heat input to both the oil, and the surrounding bath, and immersion of the samples on all occasions at the same point in the heating and cooling cycle allowed for excellent reproducibility with respect to the important variable-heat treatment.

Forewarming and concentrating in microviscometers. Samples may be forewarmed and concentrated in microviscometers. Forewarming may be conducted either in the presence of air or in vacuo. Although the procedure for concentration is not complicated, its use is recommended only under special conditions, for painstaking effort is required, samples are lost occasionally and, finally, the concentrate, because of the employment of quiescent evaporation, is not comparable with the concentrate obtained in turbulent evaporation. Special conditions which justified forewarming and concentrating in microviscometers were met, for example, in the study of additives when these were available only in very small quantities or when they were heat-labile. Appropriate formulae for calculation of the degree of concentration in terms of thread length were easily derived.

Measurement of viscosity. Viscosity measurements were made according to the method described in the preceding paper (4). It was found expedient to take as a starting point in the motion of the bead a point reasonably close to the bottom of the viscometer. Consequently, the zero point was located at approximately 0.4 cm. above the base of the thread. The time required by the bead to move first 0.75 cm., and then 0.375 cm., was noted. Two stop watches were employed for the purpose. At times the flow characteristics of milk were not adequately defined, even for practical purposes, at a single applied shearing stress, and to meet such contingencies a plastometer was constructed by means of which the applied force could be varied in steps from approximately one to 35 times gravity. This plastometer consisted of a quick-acting constant-speed motor equipped with reduction gears and pulleys driving a gear-actuated turntable. The turntable, a plastic disk 30 cm. in diameter, contained 16 radial groove-like lodgements for the viscometers. Inasmuch as the velocity of the bead moving through a truly viscous fluid is proportional at any moment to the applied centrifugal force, the viscosity of the fluid could be calculated if, in addition to the parameters associated with the viscometer, the parameters associated with the centrifuge were known. The plastometer also lent itself to the

characterization of the thixotropic behavior exhibited by concentrated milks at one stage or another of their storage life.

Storage of samples. Samples were stored in a constant temperature room maintained at 30° C.

Microviscometers were accommodated in corks with drilled spaces. The corks fitted into plastic tubes which served to shield the viscometers from accidental breakage. During storage it was found desirable to rotate the microviscometer at about 5 r.p.m., with its axis always in a horizontal position, to prevent the complicating effects of creaming and sedimentation in the study of gelation.

RESULTS AND DISCUSSION

Determination of the instrument constant. The instrument constant, if maximum precision is desired, is determined by measuring the velocity of the bead in a liquid of known viscosity and density (3). This procedure in studies on evaporated milk is too time-consuming and tedious, in view of the large number of viscometers which are employed. Fortunately, precision can be sacrificed in the interest of expediency in such studies. Because of the non-Newtonian character of sterile concentrated milk and because, in storage studies, knowledge of changes in viscosity supersede in importance accurate knowledge of the initial viscosity, that is, the viscosity of the unsterilized sample, conditions of measurements of the initial viscosity can be relaxed.

The interpretation of viscosity data on evaporated milk should be made in the light of the anomalies known to belong to these products (1, 5). The dependence of the viscosity coefficient on shearing rate, on the history of the sample, and on mechanical disturbance are well known. A single measurement made under carefully defined conditions is useful at best as an index defining an order of magnitude which can be compared with other indices obtained in the same manner and related to what may be described as the sensible body or consistency of the product.

The instrument constant may be calculated with sufficient accuracy if the two easily measured parameters, the diameters of the bead and the tube, are shown. The function $f\left(\frac{d}{D}\right)$ of the ratio between the bead and the tube diameter is calculated for various values of the ratio $\frac{d}{D}$ by means of the following equation (6):

$$f\left(\frac{d}{D}\right) = \frac{\eta_m v}{d^2 (\rho_b - \rho_m) g \cos \theta} \qquad (1)$$

η_m and ρ_m are the viscosity and density, respectively, of unsterilized evaporated milk, v is the velocity of the bead, ρ_b its density, d its diameter, g is the gravitational constant, and θ is the angle of inclination of the viscometer. The parameters on the right-hand side are easily measured. Over a limited range of $\frac{d}{D}$ values, $f\left(\frac{d}{D}\right)$ may be plotted as a linear function of $\frac{d}{D}$. This has been done in Figure 1.

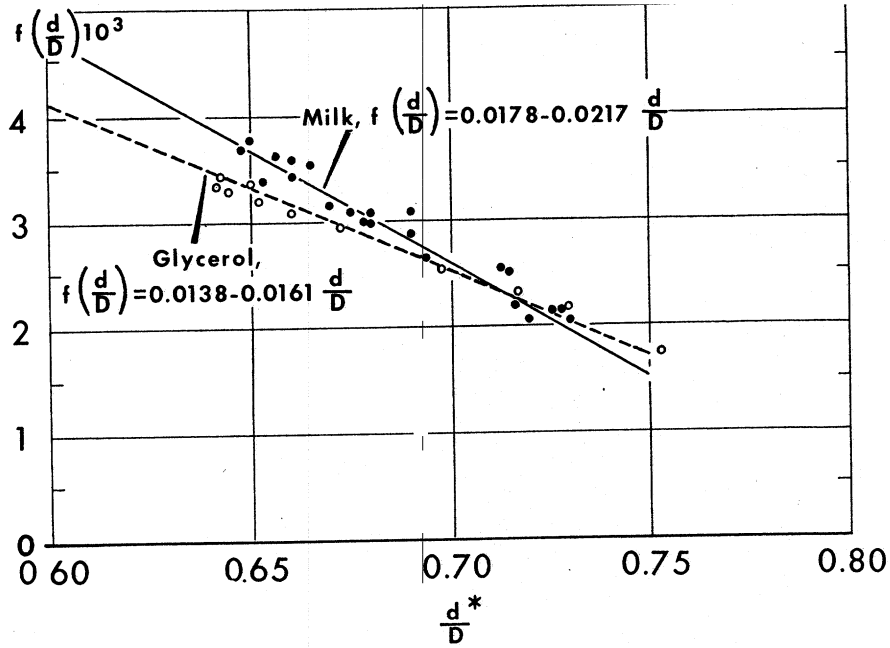

FIG. 1. The relationship over a limited range of values between the function $f\left(\frac{d}{D}\right)$ of the ratio between the bead and tube diameters and the ratios $\frac{d}{D}$ The curve referring to concentrated unsterile milk (26% solids) does not coincide with the one referring to glycerol solutions.

Two lines, the constants of which were calculated by the method of least squares, are shown; one refers to measurements on glycerol solution, the other to measurements on concentrated milk containing 26% solids. The fact that the lines do not coincide means that milk is behaving anomalously, a consequence possibly of its non-Newtonian character. To calculate the instrument constant, C_m, the value of $f\left(\frac{d}{D}\right)$ for milk corresponding to the ratio $\frac{d}{D}$ is read from the graph and inserted in Equation 2, thus:

$$C_m = f\left(\frac{d}{D}\right) d^2 g \cos\theta \qquad (2)$$

The viscosity of a milk sample contained in the viscometer is then given by Equation 3, thus:

$$\eta_m = \frac{C_m(\rho_b - \rho_m)}{v} \quad (\text{v} = \text{bead velocity}) \qquad (3)$$

Equations 2 and 3, when combined with the respective values 981 dynes, 0.983, 2.42, and 1.05 for g, cos θ, ρ_b and ρ_m substituted therein yield Equation 4, thus:

$$\eta_m = 1{,}340\, f\left(\frac{d}{D}\right)\frac{d^2}{v} \text{ (for evaporated milk with } \rho_m = 1.05) \qquad (4)$$

TABLE 1

Calculation of viscometer constant and viscosity

Tube diameter (cm.)	Ratio between diameters[a]	$f\left(\frac{d}{D}\right) \times 10^3$	$\frac{C \times 10^3}{cm^3/t^2}$	Viscosity calculated (centipoises)
0.0427	0.720	2.15	1.97	3.94
0.0427	0.715	2.28	2.06	3.50
0.0427	0.670	3.27	2.60	3.88
0.0427	0.716	2.25	2.05	3.91
Mean				3.81
Standard deviation				0.21
Viscosity (transpiration type viscometer)				3.78

[a] Ratio between bead and tube diameters.

Table 1 shows the results of an experiment in which milk of known viscosity was loaded into four viscometers varying in their instrument constants. The values of $f\left(\frac{d}{D}\right)$ were obtained from Figure 1, C was calculated by means of Equation 2, and from knowledge of these values, and the values of v and d, the viscosity of the product was calculated by means of either Equation 3 or 4. The viscosity of the milk as measured with a transpiration type viscometer operating at a maximum shearing stress of 160 dynes per cm.^{2} was 3.78 centipoises. The viscosity as measured with the microviscometer averaged 3.81 with a standard deviation of $\pm$ 0.21 centipoise. This constitutes good agreement.

Viscosity changes attending sterilization and storage. Concentrated milks sterilized in microviscometers are apt to exhibit the phenomenon of false body to a greater extent that the same milk sterilized under the conditions of turbulent flow. The following sequence of changes is usually, although not always, observed in studies with the bomb microviscometer; the viscosity first decreases (a characteristic of false body) more or less over what in some cases is a considerable period of time; it then remains measurably constant for a storage interval the duration of which is not clearly governed by the rate of change which precedes and follows; the apparent viscosity begins to rise slowly at first, then more rapidly until it becomes nonuniformly infinite throughout the sample.

False body may manifest itself as a soft gel-like structure immediately after sterilization, and under some conditions this structure, although not necessarily undesirable, may persist for long periods of time. Again, a highly thixotropic structure, one yielding to the application of mild shearing stresses, may develop immediately after sterilization and persist through the major portion of the shelf life of the product. It is understandable in view of the foregoing that the point at which the apparent viscosity becomes infinite can not be taken seriously as an end point indicating the end of the storage life of the sample.

A much more satisfactory end point becomes available if the criterion for judging deterioration is not gel formation but rather instability in body represented by rapid rate of increase in apparent viscosity.

Viscosity measurements immediately following the sterilization step are made on unstirred and stirred-out samples. Samples may exhibit at times

structure and complete or partially reversible coagulation following sterilization. Stirring-out of such samples is continued until segregation along the milk column is eliminated. A number of techniques for stirring-out are available. The position of the liquid thread may be reversed centrifugally a number of times. More drastic but more difficult to control is the technique of applying momentarily a microflame to the beaded base of the microviscometer. Localized heating violently disrupts the thread into fragments which are reunited by centrifuging. Finally, the technique of alternately freezing and thawing of the contents of the tube in an acetone-CO_2 bath followed by centrifuging to reverse the position of the liquid thread a number of times serves to stir out structure drastically.

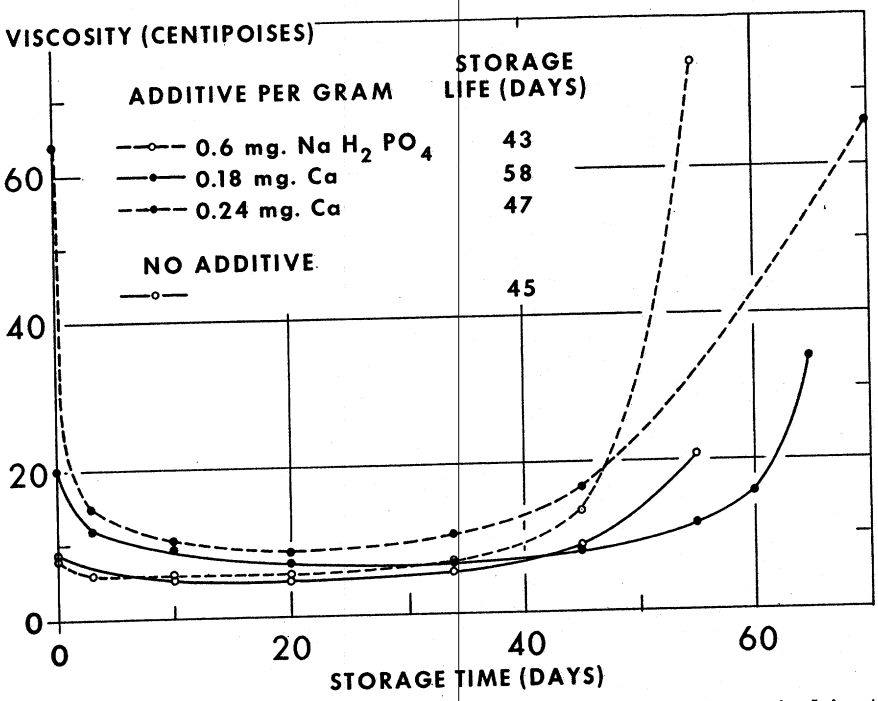

FIG. 2. Typical curves illustrating storage effects on viscosity curves obtained in studies on high temperature–short time sterilized milks with laboratory techniques employing quiescent heating and cooling. Curves also show effects of added calcium and phosphate on storage life.

Figure 2 shows a number of typical curves relating apparent viscosity and storage time. The milk employed in this study was a foam-dried product reconstituted to contain 3.5% fat and 13% total solids. It was then concentrated to 30% total solids. To each of two 1-g. samples of concentrate, 0.3 ml. of the following were added: water, 0.03 *M* $CaCl_2$, 0.04 *M* $CaCl_2$, and 0.4% Na_2HPO_4. The various samples were introduced into bomb microviscometers and sterilized for 15 sec. at 137.4° C. The viscosity of the samples prior to sterilization was 3.9 centipoises.

Although the curves in Figure 2 are intended to convey primarily some idea of the type and character of changes observed during storage (and these resemble the curves obtained in studies in which conventional techniques are employed), there are the following additional points of interest:

(1) The viscosities of the samples immediately after sterilization were related to heat stability but not to storage stability.
(2) Graininess, such as the sample containing added 0.04 *M* $CaCl_2$ showed, disappeared during storage. Such samples, therefore, could hardly be classified as defective.
(3) Both the minimum viscosity acquired by the samples, and the storage stability, were influenced only to a small degree by wide variaiton in (Ca^{++}) and $(HPO^{=}_4)$.
(4) The sample containing added phosphate showed very little false body, reached a minimum viscosity value quickly, and set once the minimum viscosity was exceeded, to a rather firm structure exhibiting macroscopically visible curd particles.
(5) The addition of $CaCl_2$ is conducive to the development of false body in proportion to the quantity added. The degree of false body reached extremely large proportions much larger than Deysher *et al.* (2) have reported for long-hold evaporated milk.
(6) The structure which developed during the storage of the high calcium concentrate was finer and possessed a higher degree of thixotropy than the structure which developed in the control, and high phosphate concentrate.
(7) The concentrates began to increase in viscosity at approximately 20 days, irrespective of whether calcium or phosphate had been added; whatever small differences in storage life were observed were related to the rate at which body developed during the period of viscosity increase.

Reproducibility of results. To obtain reproducible results, processing conditions must be adequately controlled, and viscometry conditions must be standardized. Considering the large number of variables involved, the long duration of an experiment, and the complex nature of the coagulation phenomena, one may view with satisfaction the results obtained with laboratory techniques.

The data in Table 2 are illustrative. They show the results of an experiment in which samples of concentrated milk containing 7.3% fat and 26% total solids were sterilized at 137.4° C. for 15 sec. All samples were derived from the same batch of forewarmed milk. Three concentrates, each prepared to contain the same concentration of solids, were sampled, two in duplicate and one in triplicate. Viscosities were measured before and after sterilization and during storage.

Variations between concentrates were observed corresponding to a standard deviation in presterilization viscosity of 0.1 centipoise. The possibility that variations in storage life were related to the slight variations in presterilization viscosity between samples could not be ruled out on the basis of variance analysis.

TABLE 2

ata pertaining to the storage life of samples prepared from the same batch of whole milk

Sample [a]	Viscosity before sterilization	Viscosity after	Viscosity minimum	Time to reach minimum	Storage life
	(centipoises)	*(cp.)*	*(cp.)*	*(days)*	*(days)*
A1	3.5	15.4	4.9	60	95
		13.7	4.8	58	92
A2	3.7	17.1	5.2	64	92
		17.5	4.6	64	90
A3	3.6	15.2	4.7	60	94
		17.5	5.0	64	98
		14.8	4.3	64	104
Mean	3.6	15.9	4.8	62	94
Limits for mean (95% confidence)	±0.1	±1.7	±0.3	±3	±5
Standard deviation	0.1	1.5	0.3	3	5
Correlation coefficient		0.6	0.06	0.6	0.17

[a] Samples represent three concentrates from same milk.

Relatively poor correlation as measured by the square of the correlation coefficient was observed between concentrate sample, and both storage life and minimum viscosity variations. A more marked correlation was observed between concentrate sample, and viscosity variations in freshly sterilized milk. The variations noted were not significant from a practical point of view. The mean storage life was 84 days; the limits for the mean at a 95% confidence level were ± 5 days.

Thermal history and concentration are two important variables which require careful control in order to obtain reproducible results. Milk as a biological fluid varies in composition from day to day and from place to place and, consequently, the effects of external variables are best evaluated statistically if time permits. In this respect, the laboratory techniques which have been described can contribute much to a broadening of the base of evaporated milk research, permitting thereby the accumulation of the large bodies of data required for adequate statistical evaluation.

REFERENCES

(1) Bateman, G. M., and Sharp, P. F. A Study of the Apparent Viscosity of Milk as Influenced by Some Physical Factors. J. Agr. Research, 36: 647. 1928.

(2) Deysher, E. F., Webb, B. H., and Holm, G. E. The Viscosity of Evaporated Milks of Different Solids Concentration. J. Dairy Sci., 27: 345. 1944.

(3) Höppler, F. The Eccentric Fall of Spheres Through Liquids or Gases in Cylinders. Z. tech. Phys., 14: 165. 1933.

(4) Leviton, Abraham, and Pallansch, M. J. Laboratory Studies on High Temperature–Short Time Sterilized Evaporated Milk. I. Easily Constructed Microviscometers Suitable for Containing Biological Materials During Sterilization and Storage. J. Dairy Sci., 43: 1389. 1960.

(5) Scott, Blair, G. W. A Survey of General and Applied Rheology. Pitman and Sons, Ltd., London. 1944.

(6) Weber, W. Systematische Untersuchung des Fallkugelviskosimeters mit Geneigtem Fallrohr. Kolloid-Z., 147: 14. 1956.